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Ultrasound Imaging of Breast Cancer

PI: Paul W. Erhardt

INTRODUCTION

Ultrasound imaging is useful in the detection, differential diagnosis and monitoring of treatment for many types of cancers. There remains, however, a need to improve upon the accuracy of ultrasound when deployed within the setting of breast cancer. The latter could reduce the need for more intensive diagnostic studies such as computed tomography, magnetic resonance imaging and radionuclide scans, as well as the need for other invasive, interventional procedures such as needle or open biopsy. One way to improve ultrasound imaging is to administer a chemical contrast agent that can enhance the magnitude of the ultrasound signal when in the vicinity of the cancerous tissue. One means of directing chemical agents to cancerous tissue is to incorporate molecular features that are specifically recognized by cancer cells compared to healthy cells. We intend to explore a distinct molecular address system that is known to become over-expressed in some types of human breast cancer tissue, by coupling it to another small molecule that has the potential to enhance the magnitude of the ultrasound signal. We will then test and compare the ultrasound images produced by this hybridized probe molecule in human cancer cell cultures that do and do not over-express and display this particular type of molecular recognition.

BODY

Background

Ultrasound or 'Uls' imaging is useful in the detection, differential diagnosis and treatment of many types of cancers including that of breast cancer. There remains, however, a need to improve upon the accuracy of Uls when deployed in these settings (1). One way to improve Uls imaging is to administer a chemical contrast agent (UlsCA), particularly when the latter can also be targeted toward the tissue of interest (2). Over-expression of integrin adhesion molecules on breast cancer cells destined to undergo metastasis provides an opportunity to target them by utilizing the RGD peptide motif as an address component for a given molecular cargo (3). While many of the UlsCA are gases, perfluorinated chains of eight or more carbons can also serve in this capacity (4). Although the latter appear to be well-suited as molecular cargos for targeted delivery, this type of combination remains to be explored for eventual use in the clinic.

Relevance

Enhancing the accuracy of breast cancer-related Uls would reduce the need for more intensive diagnostic studies such as computed tomography (CT), magnetic resonance imaging (MRI) and radionuclide (Rn) scans, as well as the need for other invasive, interventional procedures such as needle or open biopsy (1).

Rationale

We propose that it should be possible to improve the use of Uls to image certain types of breast cancer by administering an UlsCA consisting of a perfluorinated hydrocarbon conjugated to an RGD peptide motif.

Objectives

1. Synthesize Arg-Gly-Asp-N-CH₂CH₂(CF₂)₇CF₃ ("Model UlsCA").
2. Establish MDA-MB-435 as an in-house cell culture line.
3. Compare the Uls images of MDA-MB-435 versus MCF12A with and without the "Model UlsCA."
4. Relative enhancement of the Uls for MDA-MB-435 when the "Model UlsCA" is present will constitute a 'proof of principle' at the in vitro level and thus become the basis for a major grant submission and broader investigation of this topic.

Methods

Approximately 250 mg of the "Model UlsCA" will be prepared by using standard, solution-phase, peptide coupling reactions starting with di-protected Arg. The final product will be characterized by MS, NMR (proton and fluorine), HPLC and elemental analysis. MDA-MB-435, an estrogen-independent breast cancer cell line that is known to express high levels of the integrins (5), will be purchased and added to our panel of in-house cell cultures such as MCF12A which will be used as a comparative control since it is a non-cancer but immortalized breast epithelial cell line. Uls measurements will be done with a small unit initially designed to be used for rodents but also suitable for use with solution samples. A simultaneous or directly competing comparison will be accomplished via suspending the two cell types in solutions on opposite sides of a dialysis membrane. Side-by-side comparisons will be accomplished by using a plate/rinse off approach followed by Uls measurement. Cells types can be compared to themselves with and without the "Model UlsCA," as well as to each other with and without the "Model UlsCA."

KEY RESEARCH ACCOMPLISHMENTS & REPORTABLE OUTCOMES

Although this grant was awarded on-schedule, there was a significant delay in our undertaking of the work due to the fact that we needed to staff both the postdoctoral and graduate student participants, both of which took longer than anticipated. Thus, the project did not get underway in a really meaningful manner until early 2007. **Note that we requested a 'no-cost-extension' (NCE) near the end of the grant's initial one-year anniversary and, because of that procedure, we did not think that we needed to submit an annual report at this time.** We have since been advised that we do need to still submit this report (now past-due) and that such a report is actually needed to be granted the NCE.

In terms of progress, as assessed directly from the objectives listed above, we have now synthesized the fully-protected version of the target probe compound (i.e. its penultimate synthetic intermediate) on a scale that should be large enough to yield the amount of material that was initially desired. However, we are having significant

difficulty in establishing an ultrasound assay at the in vitro level. While we plan to continue to try to accomplish this objective, we are also now contemplating using an in vivo, tumor-implant model. We are familiar with establishing such models but have not yet tried to couple these with ultrasound diagnostics methods, although there is certainly adequate clinical precedent for deploying ultrasound in this mode. If we are forced to take this type of back-up plan for the biological assessment, then we may also need additional quantities of the probe compound since the in vivo work will require more drug than the in vitro work. We are prepared to perform these additional scale-up syntheses as needed.

CONCLUSION

Although off to a late start and having definitely run into some significant hurdles in terms of the biological assay, we are still optimistic that we will be able to accomplish the broader or overall goal of our proposed research, namely to ascertain if tumor interfaces might be better defined with ultrasound by deploying tumor specific contrast enhancement agents. Toward that end we have requested a one-year “no-cost-extension” and we plan to keep working on this project until we have at least accomplished the appropriate tests in such a fashion so as to pass judgment on the feasibility of such a diagnostic method.

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